
Lunar cycles and reproductive activity in reef fishes with particular attention to rabbitfishes

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Abstract

Cues from the moon influence synchrony in growth, feeding, migration, behaviour and reproduction of many reef fishes. Compared with comprehensive studies on the annual and daily activities of fish, few physiological studies have paid attention to the importance of lunar cues in reproductive activities. We review mutual and interesting relationships between fish reproduction and environmental changes induced by the moon, with particular emphasis on the reproductive activity of the rabbitfishes (Siganidae). Rabbitfish species exhibit, in nature, a definitive reproductive season, which differs among the tropical areas. During the reproductive season, synchronous spawning of rabbitfish is associated with a particular lunar phase. The lunar phase used by the respective species is similar in different regions on the earth. Histological observations revealed that gonads develop synchronously towards a peak around the spawning lunar phase, after which the gonads return to spent condition. Concomitant with gonadal development, sex steroid hormones were produced under the influence of gonadotropin (GtH). Injections of human chorionic gonadotropin (hCG) to the fish that are undergoing active spermatogenesis accelerated testicular maturation. These results suggest that hormonal response in maturing the gonads in rabbitfish is under the regulation of GtH, and that pituitary secretion of GtH according to the lunar cycle accounts for the lunar rhythm in gonadal development. We speculate that the cues from the moon can be recognized by the higher parts of the hypothalamus–pituitary–gonadal axis. Possible relationships between exogenous environmental factors and the lunar-reproductive rhythm are also discussed.

Keywords coral reef, lunar cycle, moon, rabbitfish, reproduction, spawning, synchronization

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Received 8 Mar 2004
Accepted 31 Aug 2004

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Introduction

The synchronization of recruitment, development and release of gametes is one of the important reproductive events in vertebrates. With a specific and regular interval, each species achieves synchronization of reproductive activity. Regular fluctuations of water temperature and photoperiod, which become marked from the temperate to the polar zones, trigger initiation and synchronization of various reproductive events in fishes. In the tropics where there is less change in photoperiod and water temperature, regional variations in environmental cues are often utilized as a forcing oscillation which entrains a biological rhythm (often called a *Zeitgeber*) by the fish for synchronization of reproductive activity. Cloud cover associated with the rainy season(s), or the flooding during the rainy season(s) may be used as a *Zeitgeber* for synchronization of reproductive activity in certain fishes living in the tropical and equatorial zones (Hyder 1970; Billard and Breton 1978; Schwassmann 1978; Boujard 1992).

Rhythmicity entrained by complex associations of the sun–earth–moon orbit also brings various environmental changes such as changes in tide and moonlight. These also have been successfully used by fish species to synchronize their reproductive activities. The importance of such environmental changes has increased relatively in reef fishes, because rhythmic variations of photoperiod and water temperature are less marked in the tropical and equatorial zones. According to Thresher (1984), reef fishes such as Acanthuridae, Apogonidae, Balistidae, Blenniidae, Carangidae, Chaetodontidae, Epinephelinae, Labridae, Lutjanidae, Mugilidae, Mullidae, Opistognathidae, Pomacentridae, Pseudochromoids, Pteroidae, Scaridae, Siganidae, Sphyrinae and Sparidae exhibit moon-related reproductive patterns. At present, however, some of the reports on a lunar-related reproductive rhythm are circumstantial or unconfirmed, because most studies have been carried out based upon field observations of spawning behaviour and on the occurrence of spawned eggs.

Diverse adaptive strategies to lunar synchronization among the reef fishes make confirmation of these reports complicated. Additionally, the lack of definitive physiological studies compounds the problems for determining lunar synchrony of reproduction.

Understanding the participation of the moon in the synchrony of biological activities in fishes is significant from the perspective of fisheries and aquaculture as well as for chronobiology. Compared with comprehensive studies on the annual and daily activities of fish, research about physiological events entrained by the lunar cycle has been limited, although the moon imposes a number of important biological rhythms on the earth. In the first part of this review, we show mutual and interesting relationships between the reproductive activities in fishes and environmental changes brought about by the moon. In the second part of this review, we focus on the characteristic nature of lunar-related reproductive activity in rabbitfish, based upon previously published reports as well as on recent results obtained from our laboratory. Rabbitfish from the family Siganidae comprises two genera, *Lo* and *Siganus*, with approximately 26 species (Woodland and Allen 1977). They are well known to show a strong synchronization in spawning with lunar events (Lam 1974; Thresher 1984; Duray 1990), and are distributed widely over coral reefs of the Indo-Pacific region (Woodland and Allen 1977), in the Red Sea and in the eastern Mediterranean (Popper and Gundersmann 1975), where rabbitfish are very important in aquaculture (Lam 1974). As rabbitfishes are resilient under experimental conditions and spawn in synchrony to the lunar cycle even under culture conditions (Hara *et al.* 1986), they are useful models for the study of mechanisms that entrain physiology to lunar cycles.

Cycles in relation to the moon

Several cyclic events are related to the position of the moon relative to the earth and sun. One category of moon-related cycles is the synodic month, which occurs at an interval of 29.53 days. It results from the orbital positions of moon, sun and

earth. The sidereal month (27.32 days) is a cycle of the moon around the earth relative to a distant star. Another moon-related cycle is repeated at 14.76 days as an interval between conjunction and opposition or 13.66 days when determined as the interval between the moon crossing the equator (Ali *et al.* 1992). Month long and fortnight long cycles are categorized as the lunar and the semilunar cycles respectively. Based on these cycles, various types of environmental phenomena change dramatically on the earth. Among cyclic environmental changes, the intensity of moonlight, the time of moonrise, relative to the solar cycle, and/or the pattern of movement of the moon across the night sky are repeated at an interval of approximately 1 month (the lunar cycle). On the contrary, the earth-moon-sun gravitational forces ('tidal' forces), or the subtle alternations in the earth's geophysical forces that occur as a result of the changing position of the earth relative to the moon and the sun are also repeated at an interval of approximately 2 weeks (the semilunar cycle). The tidal forces include rhythmic and continual changes in water level and current direction and speed (Gibson 1992). Changes in weak electromagnetic forces and radiation fields may also be involved (Leatherland *et al.* 1992). The lunar-related changes are intertwined with each other and with daily and seasonal environmental changes in habitats.

Moon-related reproductive activity

Utilization of lunar-related environmental changes has enabled the reproductive success of several teleost fishes. In the case of mature mummichog (*Fundulus heteroclitus*, Fundulidae), fertilized eggs are deposited in the salt marsh vegetation (*Spartina alterniflora*, Gramineae) or in empty shells of the Atlantic ribbed mussel (*Geukensia demissa*, Mytilidae) around the high water of the spring tide (Taylor and DiMichele 1980). Hatching occurs at a time when the eggs are immersed in water around the next series of spring tides. Similar reproductive strategies are reported in the California grunions (*Leuresthes tenuis*, Atherinidae) (Clark 1925), and a puffer fish, *Fugu niphobles* (Tetraodontidae) (Nozaki *et al.* 1976), in which the fertilized eggs are left on sandy shores and on pebble beaches respectively. It is postulated that the advantage of these spawning behaviours is related to reduction of egg predation by predators in the water. However these spawning strategies do not avoid predation from the land and

the air. Certain territorial and site-attached damselfishes, such as *Amphiprion clarkii* (Amphiprioninae) (Moyer and Bell 1976), *A. melaonopus* (Amphiprioninae) (Ross 1978), *Pomacentrus nagasakiensis* (Pomacentridae) (Moyer 1975), *P. flavicauda* (Pomacentridae), *P. wardi* (Pomacentridae) (Doherty 1983), *Microspathodon chrysurus* (Pomacentridae) (Pressley 1980), *Abudefduf saxatilis* (Pomacentridae) and *A. troschellii* (Pomacentridae) (Foster 1987) exhibit reproductive activity with peaks near the new and/or the full moon. Male *Siphaeramia orbicularis* (Apogonidae), a mouthbreeder, also release their larvae at the high tide between the first quarter and the full moon (major peak) and between the last quarter moon and the new moon (minor peak) (Allen 1972). Spawning periodicity relative to the time of high tide has been reported in wrasses, *Labroides dimidiatus* (Labridae) and *Thalassoma lunare* (Labridae) (Robertson and Choat 1974) and *T. lucasanum* (Labridae) (Warner 1982), *T. duperrey* (Labridae) (Ross 1983) and some *Haliichoeres* species (Thresher 1979). The advantage of these strategies may be to minimize immediate egg and larval predation and to facilitate their transportation to offshore locations by the strongest outgoing tide (Thresher 1984).

Neolamprologus moorii (Cichlidae) and *Lepidolamprologus elongatus* (Cichlidae) inhabiting Lake Tanganyika lay their eggs during the full moon (Rossiter 1991), although they are not reef fishes. This strategy is postulated to facilitate parental defence against the major nocturnal predators in the lake. Synchronous spawning around the specific lunar phase is observed in tanks in rabbitfishes (Hoque *et al.* 1998; Rahman *et al.* 2000c). In those cases, synchronicity of spawning occurs in places without a direct effect of tidal stimulation. Migration and aggregation to 'spawning grounds' around the specific lunar phase are reproductive characteristics in groupers, *Epinephelus guttatus* (Serranidae) (Collin *et al.* 1987; Sadovy *et al.* 1994) and *E. striatus* (Serranidae) (Collin *et al.* 1987) and a coral trout, *Plectropomus leopardus* (Serranidae) (Ferreira 1995). These examples suggest the possibility that an advantage of lunar periodicity is to synchronize gonadal maturation and to increase opportunities for encounter with appropriate partners and finally for successful spawning. Therefore, the timing of spawning around the full moon may serve better the needs of the adults rather than of their progeny (Collin *et al.* 1987). It is likely that the above-mentioned possibilities in the utilization of lunar

cues are closely related to the adaptive strategies of each fish species (Takemura *et al.* 2004).

Very few studies have investigated lunar synchronization in teleost fishes from physiological aspects. The only series of studies has been performed on mummichog (Taylor *et al.* 1979; Taylor and DiMichele 1980; Bradford and Taylor 1987; Hsiao and Meier 1989; Hsiao *et al.* 1994), in which the gonadosomatic index (GSI) during the reproductive season is higher during the spring tide periods than between the spring tides (Taylor *et al.* 1979). Maturation of primary oocytes starts within a few days after the previous spawning, which occurs at spring tide, and yolk accumulation into the oocytes accelerates towards the next spring tide (Taylor and DiMichele 1980). Coincident with GSI and gonadal maturation, serum and ovarian fluid oestradiol-17 β (E₂) and serum corticosteroid hormone levels change with peaks preceding the maximal gonadal size (Bradford and Taylor 1987). Mummichog retains a semilunar periodicity of spawning in habitats that provide little or no tidal stimulus (Hines *et al.* 1985) and in aquarium with constant conditions (Hsiao and Meier 1989). Interestingly, Simizu (1997) reported that the laboratory-reared strain of this species exhibits daily spawning activity but not semilunar spawning. It is possible that the semilunar spawning rhythm in this species has been lost by generations reared under culture conditions (Simizu 1997). Spawning rhythms of this species can be entrained by artificial moonlight, day–night regimes and tidal periodicity of water movement, indicating that although there is an apparent endogenous rhythm of gonadal maturation in this species, they are responsive to a variety of Zeitgebers that can synchronize the rhythm (Leatherland *et al.* 1992). This seems to be completely different from rabbitfishes that strictly repeat gonadal maturation and spawning with the lunar cycle (see below).

Reproductive season and lunar-related spawning rhythm of rabbitfishes

Many studies have been carried out on the reproductive season of rabbitfishes in tropical and the subtropical waters. The initiation and termination of reproductive season has been determined by following changes in gonadal histology, GSI and spawning activity. The reproductive season of the forktail rabbitfish (*Siganus argenteus*, Siganidae), which inhabits the waters around the Ryukyu

Islands, Japan, lasts for 3 months from May to July (Salaki 1993). In the same region, a maximal GSI is maintained for 3 months from May to July in the seagrass rabbitfish (*S. canaliculatus*, Siganidae) (Hoque *et al.* 1998) and the spiny rabbitfish (*S. spinus*, Siganidae) (Harahap *et al.* 2001), and in June and July in the golden rabbitfish (*S. guttatus*, Siganidae) (Rahman *et al.* 2000a,b). When comparing a reproductive season of these three rabbitfish species in different tropical regions, the forktail rabbitfish has the reproductive season from February to September in the Philippines (Luchavez and Carumbana 1982) and from May to June in Micronesia (Park 2004). The major reproductive season of the seagrass rabbitfish lasts from January to April in Singapore (Soh and Lam 1973), from April to August in Palau (Bryan *et al.* 1975), from March to May in the Arabian Gulf (Al-Ghais 1993; El-Sayed and Bary 1994) and from March to June in Hong Kong (Tseng and Chan 1982). A second or minor reproductive season is found for some species. Except in the Arabian Gulf, the occurrence of the minor reproductive season is seen in the seagrass rabbitfish inhabiting other regions (Soh and Lam 1973). In nature, each rabbitfish exhibits a definitive reproductive season, which differs by tropical region. Thus, it appears that reproductive seasonality is affected by regional variation of environmental factors such as water temperature and photoperiod, and food abundance. However, a year-round spawning has been achieved for the captive golden rabbitfish in the Philippines, so long as a lipid-enriched diet was given to the captive fish (Hara *et al.* 1986). Lam (1974) indicated that seagrass rabbitfish will mature in captivity, as assessed by milt release, earlier than in nature when kept in tanks with low nitrite level, a pH of 7.8 ± 0.1 , water temperature 26–30 °C, adequate aeration and water circulation, and fed with chicken food pellets. It is thus possible that the duration of the reproductive season of rabbitfish can be protracted, to some extent, according to particular environmental and nutritional conditions (Tawata 1988). Further, injections of human chorionic gonadotropin (hCG) could also induce spawning of the seagrass rabbitfish in the Philippines throughout the year (Bryan *et al.* 1975).

During the reproductive season, the gonad of forktail rabbitfish in the coastal waters of the Ryukyu Islands develops towards the last quarter moon and diminishes rapidly at the new moon (Fig. 1). A similar spawning pattern is known for

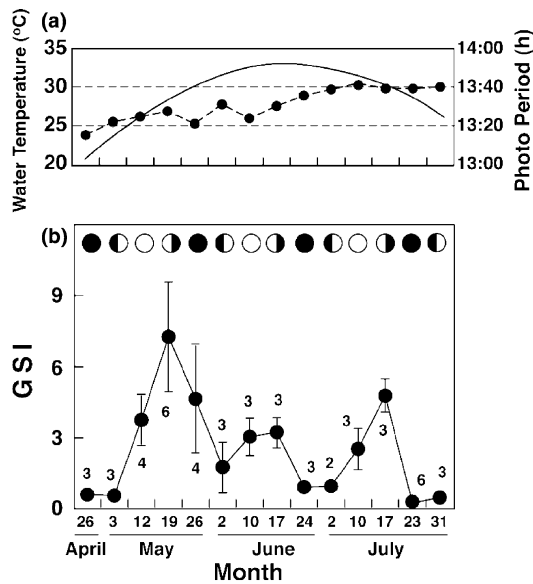


Figure 1 Weekly changes in water temperature and photoperiod (a), and gonadosomatic index (GSI) of female *Siganus argenteus* (b) during the reproductive season. Each GSI value represents the mean \pm SEM. The number of fish sampled is also indicated. Moon phases are shown as ●, new moon; ◐, first quarter moon; ○, full moon and ◑, last quarter moon (Rahman *et al.* 2003c).

the forktail rabbitfish of the Philippines (Burgan and Zselezky 1979; Luchavez and Carumbana 1982) and Micronesia (Park 2004). In the case of the seagrass rabbitfish, synchronous spawning is reported after the new moon in the Ryukyu region (Tawata 1988; Hoque *et al.* 1999) and in Palau (Hasse *et al.* 1977). The synchronous spawning of the golden rabbitfish is observed around the first quarter of the moon in the Ryukyu region (Rahman *et al.* 2000c,d) and in the Philippines (Harvey *et al.* 1985; Hara *et al.* 1986). This lunar phase is also utilized by the vermiculated rabbitfish *S. vermiculatus*, Siganidae) in Fiji (Popper *et al.* 1976). It is likely that lunar Zeitgebers recognized by respective species are common in any habitat regions on earth, although the lunar phase for timing of synchronous spawning is different among rabbitfishes. However, under captive conditions, handling the fish (Bryan *et al.* 1975) and transferring the fish into tanks with lower water level, induced spawning in the forktail rabbitfish (McVey 1972). The introduction of fresh seawater to holding tanks brought prompt spawning of marbled spinefoot (*S. rivulatus*, Siganidae) within 10 h (Popper *et al.* 1973). These findings suggest that siganids respond to various stimuli and

stressors with spontaneous spawning. However, it seems that only mature individuals approaching the expected spawning lunar date can respond to such environmental changes, because gonads develop synchronously in conjunction with the approach of a particular phase of the moon and matured gametes are observed only around the lunar phase appropriate for spawning (see below).

Ovarian development and the lunar cycle

Weekly changes in rabbitfish ovaries during the reproductive season have been observed histologically and they exhibit the characteristics of a lunar-synchronous rhythm (Hoque *et al.* 1999; Rahman *et al.* 2000c; Harahap *et al.* 2001). In the case of female forktail rabbitfish, which show a group synchronous ovarian development, only immature oocytes at the chromatin-nucleolus and the perinucleolus stages were observed in an ovary during the non-reproductive season (Salaki 1993). With the onset of the reproductive season, oocytes accumulating oil-droplets in the cytoplasm (the oil-droplet stage) appeared among the immature oocytes, and oocytes laden with yolk increased in number and size from the time of the new moon (Fig. 2a) to the first quarter moon (Fig. 2b). Oocytes at the tertiary yolk stage and the maturation stage were predominant in the ovary at the full moon (Fig. 2c) and the last quarter moon (Fig. 2d) respectively. Ovulatory follicles, which are a conventional indicator of spawning (Rahman *et al.* 2000c), were observed among oocytes at yolk stages in the ovary around the last quarter moon (Fig. 2e), suggesting that spawning occurred between the full and the last quarter moon. These histological indications of ovarian development during the lunar periodicity show that yolk incorporation into developing oocytes is active from the first quarter moon to the full moon (vitellogenic stage), and then the oocytes are under maturation conditions around the last quarter moon (final oocyte maturation stage). A similar oocyte development at one lunar monthly interval has been reported in the seagrass rabbitfish (Hoque *et al.* 1999), the golden rabbitfish (Rahman *et al.* 2000c) and the spiny rabbitfish (Harahap *et al.* 2001).

Certain hormones directly or indirectly participate in gonadal development in fishes. It is known that E_2 and $17\alpha,20\beta$ -dihydroxy-4-pregnen-3-one (DHP), which are produced in the ovarian follicles under regulation of gonadotropin (GtH), stimulates

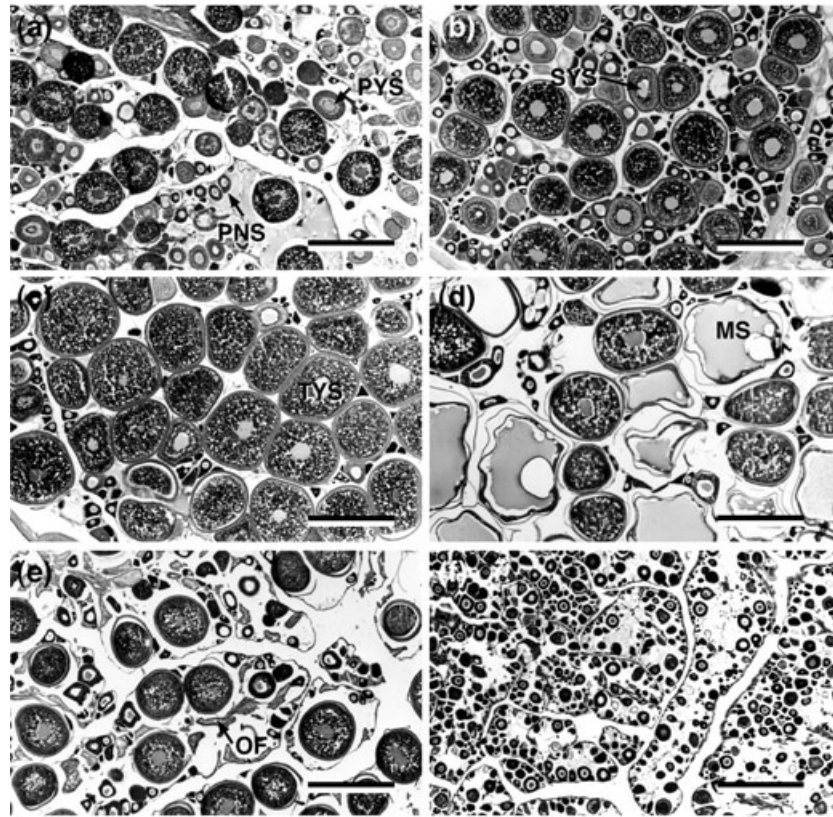


Figure 2 Histological observations of ovaries in *Siganus argenteus* according to the lunar cycle, (a) cross section (CS) of an ovary on 26 May (new moon), showing small numbers of oocytes at the primary to tertiary yolk stages, (b) CS of an ovary around the first quarter moon, showing oocytes at the peri-nucleolus stage together with the primary to tertiary yolk stages, (c) CS of an ovary around the full moon, showing many oocytes at the tertiary yolk stage, and a few oocytes at primary and secondary yolk stages, (d) CS of an ovary around the last quarter moon containing a large number of eggs at maturation stage together with tertiary yolk stage, (e) CS of an ovary around the last quarter moon, showing abundant ovulatory follicles with small numbers of oocytes at the secondary and tertiary yolk stages, (f) CS of an ovary on 23 July (new moon), showing mostly oocytes at the peri-nucleolus stage. PNS, peri-nucleolus stage; PYS, primary yolk stage; SYS, secondary yolk stage; TYS, tertiary yolk stage; OF, ovulatory follicle; MS, maturation stage. Scale bar = 300 μ m. For preparation of histological samples, ovaries were collected weekly and subjected to histological observation, according to the method of Hoque *et al.* (1999).

hepatocytes to produce vitellogenin, a female-specific protein (Wallace and Selman 1981), and final oocyte maturation (Nagahama 1994) respectively. It is considered that testosterone (T) is a precursor form of E_2 , and is synthesized in the theca cells and then aromatized in the granulosa cells during vitellogenesis. 17α -hydroxyprogesterone (17α -OHP) is converted to DHP by 20β -hydroxysteroid dehydrogenase (20β -HSD) during final oocyte maturation (Nagahama 1994). When plasma levels of the steroid hormones were measured in the forktail rabbitfish with regard to the lunar cycle, E_2 and T concomitantly increased from the first quarter

moon to the full moon and decreased around the last quarter moon (vitellogenic stage). Alternatively, DHP peaked around the last quarter moon (final oocyte maturation stage) (Fig. 3). Fluctuation of the plasma steroid hormones was reported in the golden rabbitfish, in which E_2 , T and DHP increased towards and decreased rapidly after the first quarter moon (Rahman *et al.* 2000c). *In vitro* incubation of ovarian pieces from the golden rabbitfish revealed that E_2 and DHP productions into the medium are stimulated under the influence of hCG around the new moon and the first quarter moon respectively (Rahman *et al.* 2002). T and 17α -OHP were

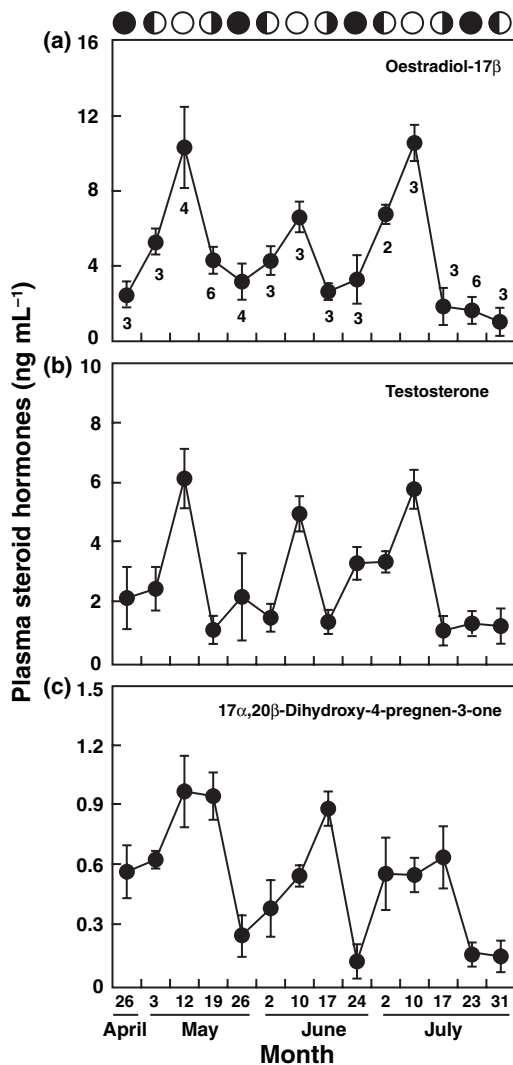


Figure 3 Weekly changes in (a) oestradiol-17 β , (b) testosterone and (c) 17 α ,20 β -dihydroxy-4-pregnen-3-one in the plasma of female *Siganus argenteus* according to the lunar cycle. Each value represents the mean \pm SEM. The number of fish sampled is indicated. Moon phases are indicated as ●, new moon; ○, first quarter moon; ○, full moon and ○, last quarter moon. Plasma samples were collected weekly during the reproductive season and subjected to hormonal measurement by enzyme-linked immunosorbent assay, according to the method of Rahman *et al.* (2000c).

converted to E₂ and DHP by the cultured ovarian pieces during the new moon and the first quarter moon respectively. A high level of final oocyte maturation was induced *in vitro* by the addition of DHP as well as 17 α ,20 β -21-trihydroxy-4-pregnen-

3-one (20 β -S) into the medium around the time of the first quarter moon, but not of the new moon (Rahman *et al.* 2002). These results suggest that E₂ and DHP/20 β -S are synthesized in the ovarian follicles during the stages of vitellogenesis and final oocyte maturation respectively, and play important roles in reproductive phases in the rabbitfishes.

Successful spawning has been induced in the vermiculated rabbitfish by a single injection of hCG at 0.3 IU g⁻¹ BW (Popper and Gundermann 1976) and in the golden rabbitfish by two injections of hCG at 2 IU g⁻¹ BW (Ayson 1991). Injection of luteinizing hormone-releasing hormone analogue (LHRHa) also induced spawning of the vermiculated rabbitfish (Popper *et al.* 1976). Harvey *et al.* (1985) reported that the spawning of the sea bass (*Lates calcarifer*, Centropomidae), but not the golden rabbitfish, occurs within 2 days after the implantation of LHRHa. Its implantation to the golden rabbitfish was effective in synchronous spawning around the expected date of spawning as predicted by the lunar date (Harvey *et al.* 1985). However, synchronous spawning was confirmed in the vermiculated rabbitfish without hormone treatment which served as a control (Popper and Gundermann 1976; Harvey *et al.* 1985). Popper and Gundermann (1976) assumed that the critical factor for the occurrence of spawning is the right day of the lunar month. Therefore, attempting hormone treatment for purposes of inducing spawning may be effective only around the lunar phase when spawning occurs, which is when the oocytes are fully matured.

Testicular development and lunar periodicity

As for the ovaries, testicular development of rabbitfishes is closely related to the lunar cycle during the reproductive season (Rahman *et al.* 2000d, 2001, 2003a,b; Harahap *et al.* 2002). During the non-reproductive season, the testis of rabbitfishes remains immature and largely contains spermatogonia (Rahman *et al.* 2000d, 2003a; Harahap *et al.* 2002). Histological observations revealed that once the reproductive season started, spermatocytes, spermatids and spermatozoa appeared in the testis. The percentage of spermatozoa in the testis increased rapidly from the appearance of the last quarter moon to the new moon in the spiny rabbitfish (Harahap *et al.* 2002) and from the full moon to the last quarter moon in the forktail rabbitfish (Rahman *et al.* 2003a). Sperm motility of the forktail rabbitfish was low from the new moon

to the full moon but increased rapidly around the time of the last quarter moon (Rahman *et al.* 2003b). Concomitant with the acquisition of sperm motility around the spawning lunar phase, the pH and osmolality of seminal fluid increased and decreased respectively. These reports suggest that the testis of forktail rabbitfish develops rhythmically according to the lunar cycle and undergoes active spermatogenesis from the new moon and afterwards, spermiation occurs rapidly around the time of the last quarter moon. Changes occurring in the seminal fluid have been reported in the testicular maturation of many teleost fishes. The characteristic nature of rabbitfishes is that the testis develops in synchrony with the lunar cycle and sperm maturation occurs just after the lunar phase at which spawning occurs.

It is known that under the regulation of GtH, 11-ketotestosterone (11-KT) and DHP influence the progress of spermatogenesis and spermiation respectively (Nagahama 1994). Rahman *et al.* (2003a) reported that in the male forktail rabbitfish, plasma levels of 11-KT/T and DHP peak around the full moon when spermatogenesis occurs, and at the last quarter moon when spermiation is observed. HCG stimulated *in vitro* production of 11-KT in the testicular fragments of forktail rabbitfish around the full moon but not around the last quarter moon (Rahman *et al.* 2003b). When the testicular fragments and the sperm preparations were each incubated with T and 17 α -OHP, the conversion of T to 11-KT by the testicular fragments decreased and, alternatively, that of 17 α -OHP to DHP in the sperm preparations increased from the appearance of the full moon to the last quarter moon. 20 β -HSD, which is a key enzyme to convert 17 α -OHP to DHP, was activated rapidly close to the specific lunar phase. Furthermore, Rahman *et al.* (2003b) found that administration of hCG or DHP to fish prior to the lunar day, at which spawning was predicted to occur, increases sperm motility and the pH of the seminal fluid. Additionally, plasma DHP levels, but not T and 11-KT, increased after injection of hCG. It is known that these hormones accelerate testicular maturation in salmonid fish (Ueda *et al.* 1985; Miura *et al.* 1992) and goldfish (*Carassius auratus*, Cyprinidae) (Ueda *et al.* 1985). Our result suggests that hormonal response of the maturing testis of this species is under the regulation of GtH and testicular maturation is not related to changes in the lunar factors directly (Rahman *et al.* 2003a,b). It is speculated that secretion of endogenous GtH from

the pituitary in synchrony with the lunar cycle causes an apparent lunar rhythm in testicular development and that the cues from the moon can be recognized by the higher parts of the hypothalamus–pituitary–gonadal axis.

Environmental factors regulating lunar-related reproduction

To date, it is difficult to specifically identify which environmental factors affect annual reproductive activity in rabbitfishes. However, latitudinal and regional comparisons between reproductive season and environmental factors may provide an insight into this subject. When the gonadal development was considered relative to changes in water temperature and photoperiod, the initial development of the gonads coincided with an increase in water temperature as well as photoperiod (Fig. 1). Developing oocytes were not observed in the ovary in late July (Fig. 2f), when the photoperiod was decreasing even when water temperature was still high. Lam and Soh (1975) reported that a long photoperiod (18:6, L:D) retards gonadal maturation in the seagrass rabbitfish compared with the normal photoperiod (12:12, L:D). On the contrary, Tawata (1988) noted that in the seagrass rabbitfish inhabiting the Okinawa region, Japan, spawning each year starts when water temperature reaches 20 °C. Water temperature averaged 30 °C in Palau (Bryan *et al.* 1975) and 21 °C in the Arabian Gulf (El-Sayed and Bary 1994), where the natural spawning of seagrass rabbitfish were observed. Many atretic oocytes at the yolk stages are observed histologically in some seagrass rabbitfish in August (Hoque *et al.* 1999) in the Okinawa region, where water temperature is still high and equivalent to the temperature required for active spawning in Palau. It is possible that the duration of the photoperiod and its regular change are related to the initiation and termination of reproductive activity in each year. However, the involvement of water temperature in reproductive activity cannot be ruled out, especially, in higher latitudes, because water temperature in the Okinawa region and the Arabian Gulf during the non-reproductive season falls under 20 °C. Additionally, it is possible that regionally specific variations of season such as plankton productivity, which influences food availability, rainfall, which can change salinity in coastal areas and speed of prevailing currents and winds are related to the initiation and termination of reproductive activity

(Johannes 1978). Additional experimental evidence is needed to clarify the relative importance of environmental cues in reproductive activity in the tropical and the subtropical regions.

Once exogenous environmental factors become suitable for the fish, gonadal maturation and spawning are repeated at an interval of approximately 28 days according to the lunar cycle. It is worth noting that rabbitfish larvae also follow a lunar rhythm (Popper *et al.* 1976; Gundermann *et al.* 1983; Tawata 1988; Duray 1990). The shoreward migration of the newly metamorphosed fry of vermiculated rabbitfish occurs before or on the night of the new moon (Gundermann *et al.* 1983). It is reported that large groups of pelagic juveniles of fork-tail, spiny and seagrass rabbitfishes settle in coral reef areas during the flood tide after the new moon (Kishimoto 1984; Tawata 1988). This evidence suggests that the degree of maturity is not an important factor influencing the perception of lunar cues. In other words, the rabbitfish can always perceive cues from the moon to adjust endogenous rhythms (circalunar or circalunidian rhythm). However, it is a matter of course that lunar cues still play an important role in allowing synchrony of maturation.

Closing remarks

There are several reports that rhythmic changes in cues from the moon influence the growth, feeding, migration and behavior in fishes. We were not concerned here with these points, because in this review we have focused only on reproductive activity in fishes that is synchronized with phases of the moon. The cues provided by lunar events have yet to be ascertained. Few studies have evaluated the nature of the cue or cues from the moon perceived by the fish and how they might be conveyed as endogenous stimuli in endocrine organs by the fish. The stimuli derived from the phases of the moon may be linked closely to endocrine stimuli, which are capable of entrainment. Recently, we examined melatonin levels in the plasma of golden rabbitfish. Melatonin is an indolamine synthesized mainly in the pineal organ and retina, and is a phylogenetically conserved molecule involved in both photoperiodicity and coupling of circadian clocks (Cassone 1998). It is considered that, in general, nocturnal increase and diurnal decrease in the circulating melatonin level are considerable and that through changes in photoperiod, melatonin is involved in synchronization

of daily and seasonal events of behaviour and physiology in fishes (Zachmann *et al.* 1992; Bromage *et al.* 2001). As a result, the plasma melatonin levels of golden rabbitfish changed not only with photoperiod but also with intensity of moonlight; plasma melatonin level at the new moon was higher than that at the full moon (Takemura *et al.* 2004). This result may mean that the rabbitfish can perceive changes in moonlight intensity and melatonin acts as a neural and chemical transducer of environmental information signalled by moonlight. It is expected that some fishes showing activity that is synchronized with the moon mainly utilize changes in moonlight intensity, although synchronization of reproduction with lunar rhythms is thought not to be simply a passive response to its rhythmic change.

Acknowledgements

The authors wish to express gratitude to Prof. M. Nakamura, Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, for his useful advice and encouragement. Thanks are due to Prof. John F. Leatherland, University of Guelph, Canada, for critical reading this manuscript and useful suggestions. This study was supported in part by a Grant-in-Aid for Scientific Research (B) (15405029) and (C) (14560158) from Japan Society for the Promotion of Science (JSPS) to AT, and by a JSPS Research Fellowships grants for Young Scientists to MSR, and contribution from the Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, Okinawa, Japan.

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